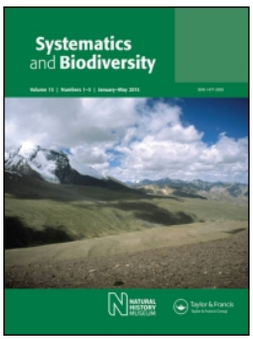

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Research Article

Phylogenetic relationships within the New World subfamily Larreoideae (Zygophyllaceae) confirm polyphyly of the disjunct genus *Bulnesia*

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The Larreoideae subfamily is the major representative of the family Zygophyllaceae in South America, where several of its members are common to dominant in arid regions of the Southern Cone. However, there are currently no phylogenetic analyses of the subfamily that may help to understand its origin and diversification. Additionally, there are taxonomic discrepancies around *Bulnesia* Gay (1845), one of its more important genera. Accordingly, we performed a phylogenetic analysis combining chloroplast (*rbcL* and *trnL-F*) and nuclear (ITS) DNA sequences. Bayesian and Parsimony analyses were performed to highlight the intergeneric relationships within Larreoideae. All genera with the exception of *Bulnesia* are monophyletic and we propose to redefine *Bulnesia*, dividing it in two genera. Furthermore, other taxonomic issues of the remaining genera are solved. This study represents the first approximation to clarify the phylogenetic relationships amongst all Larreoideae genera, producing a phylogenetic framework that can be used in future macro-ecological studies.

Keywords: arid environments, Bayesian inference, *Bulnesia*, *Gonopterodendron*, incongruences, lectotypifications, molecular analyses, phylogeny, South America, Southern Cone, taxonomy, Zygophyllaceae

Introduction

Zygophyllaceae *s.l.* is a widespread family of 22 genera and 230–240 species, characteristic of tropical, subtropical, and temperate arid to semi-arid regions worldwide (Sheahan, 2007). Members of the family are found in some of the driest regions of Europe, Asia, Australia, Africa, and America (Sheahan, 2007). Dated molecular phylogenetic analyses suggest that the diversification of Zygophyllaceae is related to arid phases from the Oligocene and onwards in Asia (Wu et al., 2015) as well as in Africa (Bellstedt, Galley, Pirie, & Linder, 2012). Semi-arid to arid environment may indeed be the ancestral climate envelope in the family, as the sister-group of the family, Krameriaceae, also consists of species that are restricted to warm semi-arid or arid environments of North and South America (Giannini, Takahasi, Medeiros, Saraiva, & Alves-dos-Santos, 2011; Simpson, Weeks, Helfgott, & Larkin, 2004). Zygophyllaceae is furthermore one of the 19 angiosperm families that have acquired C4 photosynthesis (Sage, Christin, & Edwards, 2011), a modification of the ancestral C3 pathway, that increases productivity in CO_2-depleted, hot, and water-stressed habitats.

Currently, five Zygophyllaceae subfamilies are recognized: Zygophylloideae, Seetzenioideae, Tribuloideae, Morkillioideae, and Larreoideae (Sheahan & Chase, 1996, 2000); the last being the major representative of the family in South America (Sheahan, 2007) and the topic of the present study. Within Zygophyllaceae, Larreoideae is sister to Zygophilloideae which is of African origin (Bellstedt et al., 2012). The divergence of these two subfamilies appears to have occurred during the Eocene and Larreoideae appears to have diversified during the Miocene (Wu et al., 2015). However, phylogenetic studies of Larreoideae are limited to infrageneric analyses of *Guaiacum* (Dertien & Duvall, 2014), *Larrea* (see Lia, Confalonieri, Comas, & Hunziker, 2001), and *Bulnesia* (Comas, Confalonieri, & Hunziker, 1998) – while the phylogeny of the subfamily itself is unknown.

Larreoideae has been recognized as a well-supported monophyletic group (Sheahan & Chase, 1996, 2000) comprising 27 species known for their medicinal or economic value (Sheahan, 2007) and hence of conservation interest (Dertien & Duvall, 2014). There are seven well-recognized genera in Larreoideae: *Bulnesia* (9), *Guaiacum* (5),

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Larrea (5), *Metharme* (1), *Pintoa* (1), *Plectrocarpa* (2), and *Porlieria* (4). Presently, most of these genera occupy some of the driest ecoregions of South America mainly the Monte Desert, the Patagonian steppe, and the Dry Chaco all found in the Southern Cone of South America. The Larreoideae species often form conspicuous members of the communities, where species from the genera *Larrea* and *Bulnesia*, in particular, can be dominating in the landscape to an extent that traditional biogeographers have used members of these genera as indicators of phytogeographic divisions of the Southern Cone (e.g., Cabrera, 1976). *Bulnesia* comprises nine species distributed into two subgenera (or sections) based on morphological (Crisci, Hunziker, Palacios, & Naranjo, 1979; Grisebach, 1879; Palacios & Hunziker, 1984 – but see Descole & O'Donell, 1943; Descole, O'Donell, & Lourteig, 1940) and biochemical differences. Comas et al. (1998) constructed an infrageneric phylogeny of *Bulnesia*, based on seed protein data. The authors confirmed the systematic scheme dividing the genus into two groups. However, no other Larreoideae species were included in the study that was rooted by a hypothetical all-zero outgroup. Therefore, at present, no study has tested the monophyly of *Bulnesia*.

The aim of the present study was to provide a complete and well-supported phylogeny of Larroideae that can be used as a framework in taxonomic and future macro-ecological studies. The time lag between the origin and diversification of Larreoideae (from Eocene to Miocene – see Wu et al., 2015) suggests that the subfamily may be one of the ancient arid-adapted Southern Cone taxa that persisted in local arid environments and later diversified when the open vegetation became dominant. The age and current dominance of Larroideae in some of the most extensive ecoregions of the Southern Cone furthermore imply that a better knowledge of the phylogeny of the subfamily could provide information on the origin of the flora in these ecoregions as well.

Materials and methods

Taxon sampling

The subfamily Larreoideae includes 27 accepted species names (The Plant List, http://www.theplantlist.org/). We analysed DNA sequence data of 23 Larreoideae species using the molecular markers: the *rbcL* gene, the internal transcriber spacer ITS, and the *trnL-F* region (see Appendix 1, see online supplemental material, which is available from the article's Taylor & Francis Online page at https://doi.org/10.1080/14772000.2018.1451406). All available sequence data of Larreoideae and other Zygophyllaceae subfamilies were downloaded from GenBank and included in the study (Dertien & Duvall, 2014; Lia et al., 2001; Muscarella et al., 2014; Sheahan & Chase, 1996, 2000; Appendix 1, see supplemental material online). We obtained sequences from GenBank of the following species: *Bulnesia arborea*, *Guaiacum angustifolium*, *G. coulteri*, *G. officinale*, *G. sanctum*, *G. unijugum*, *Larrea tridentata*, *Plectrocarpa tetracantha*, *Pintoa chilensis*, and *Porlieria chilensis*. The *rbcL*, ITS, and/or *trnL-F* data lacking for above mentioned species, were completed when possible (Appendix 2, see supplemental material online). Further, as part of this study, we added new sequence data for nine species not analysed in previous studies: *Bulnesia bonariensis*, *B. chilensis*, *B. foliosa*, *B. retama*, *B. sarmientoi*, *B. schickendantzii*, *Metharme lanata*, *Plectrocarpa rougesii*, and *Porlieria microphylla* (Appendix 2, see supplemental material online). In total, seven of the nine accepted *Bulnesia* species names were sampled (The Plant List, http://www.theplantlist.org/). Details of the vouchers and GenBank accession numbers of these species are shown in Appendix 2 (see supplemental material online).

Leaf material of *Porlieria microphylla* and *Bulnesia sarmientoi* were obtained from specimens cultivated at the Botanical Garden of the Buenos Aires University and from a vivarium in Salta, respectively. Furthermore, leaf material of *Bulnesia chilensis*, *B. retama*, *B. schickendantzii*, *Plectrocarpa rougesii*, and of four *Larrea* species (*L. ameghinoi*, *L. cuneifolia*, *L. divaricata*, and *L. nitida*) were obtained from field collections. All voucher specimens were deposited at the herbarium of the Botanical Institute Darwinion (SI). Material of *Bulnesia bonariensis* and *B. foliosa* was provided by the Cordoba herbarium (CORD), and material of *Metharme lanata* was provided by the Concepcion University in Chile (CONC).

Krameria was used to root the phylogeny, as it appears as the sister of Zygophyllaceae in a well-supported clade within the Eurosides I (Giannini et al., 2011; Savolainen et al., 2000; Simpson et al., 2004).

DNA extraction, PCR amplification, and sequencing

Total DNA was extracted from leaves of plants collected in the field and dried in silica gel using a modification of the Cetyltrimethylammonium Bromide (CTAB) protocol of Doyle and Doyle (1987). A DNeasy Plant Mini Kit was used when fresh material was not available, and herbarium specimens were analysed. All samples were amplified from total genomic DNA by the polymerase chain reaction (PCR). The *rbcL* gene was amplified with the primers 1F and 1351R and, in some cases, the amplification of two overlapping sections with the internal primers 660F and 675R. Amplification of ITS-*1* and ITS-*2* was performed with the primer pair ITS-4/ITS-5 (White, Bruns, Lee, & Taylor, 1990) while for *trnL-F* we used the primers *c* and *f* (Taberlet, Gielly, Pautou, & Bouvet, 1991).

PCR reactions were performed in 25 μL final volumes with 50–100 ng of template DNA, 0.5 μm of each primer, 0.5 μm dNTP, 2 μm $MgCl_2$, 2.5 μm buffer and 0.3 units of Taq polymerase provided by Invitrogen Life Technologies. In species for which these protocols were unsuccessful, additives and enhancing agents (bovine serum albumin, dimethyl sulphoxide) were used to increase the yield, specificity, and consistency of PCRs. The reaction conditions were: a first period of denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 60 s, and extension at 72°C for 90 s. Final extension at 72°C for 6 min terminated the reactions. A negative control with no template was included for each series of amplifications to check for contamination. PCR products were run out on a 1% TBE agarose gel stained with SYBR Safe DNA gel stain (Invitrogen) and visualized in a blue light transilluminator. Sequencing reactions were performed by Macrogen using the ABI PRISM BigDye Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (Applied Biosystems, Seoul, Korea) following the protocols supplied by the manufacturer. Both forward and reverse strands were sequenced with a minimum overlap of 90% for every taxon. Single-pass sequencing was performed on each template using selected primers to complete a bidirectional contig of the full sequence. Editing and assembling of sequences was conducted using Chromas Pro ver. 1.34 (http://www.technelysium.com.au/ChromasPro.html). Quality of sequences was assessed by visual inspection of the chromatograms. Sequences were manually aligned using BioEdit ver. 5.0.9 (Hall, 1999), adding gaps to the matrix. Each base-pair gap was treated as missing data.

Phylogenetic analyses

The DNA sequence data was analysed using Maximum Parsimony (MP) and Bayesian Inference (BI). Each marker was analysed individually and combined (chloroplast+nuclear).

Model-based analyses were performed with Bayesian inference (BI) using MrBayes v. 3.2.3 (Huelsenbeck & Ronquist, 2001; Ronquist et al., 2012) through the Cyber infrastructure for Phylogenetic Research (Cipres Science Gateway – Miller, Pfeiffer, & Schwartz, 2010). The best-fitting nucleotide substitution model for each marker was the general time reversible substitution model with invariable sites and discrete gamma rate heterogeneity (GTR+I+G), chosen by using the Akaike information criterion, as implemented in jModelTest 2.1.1 (Darriba, Taboada, Doallo, & Posada, 2012). Individual and combined BI analyses were set in MrBayes as nst = 6 and rates = invgamma, with rate-matrix parameters, state frequencies, and gamma shape parameter unlinked across partitions for cpDNA and ITS. Four Markov chains were run simultaneously in two independent runs starting with a random tree. The analyses were run for 10 million generations, and trees were sampled every 1,000 generations. Convergence diagnostics for log-likelihood values of the trees produced by each run were assessed visually using Tracer v.1.5.0 software (http://.beast.bio.ed.ac.uk/Tracer; Rambaut & Drummond, 2009) verifying that effective sample size (ESS) for all parameters was over 400. After discarding the initial 2500 trees of each run as burn-in, the remaining trees (15,002) were summarized in a Maximum Clade Credibility Tree (MCCT) including the posterior probabilities (PP) as branch support estimates.

Phylogenetic analysis using Maximum parsimony were conducted using TNT ver. 1.1 (Goloboff, Farris, & Nixon, 2008). All characters were equally weighted, treated as unordered, and gaps were scored as missing data. The searches used 1,000 replicates, each of which generated 10 Wagner trees using a random addition sequence of taxa from the data matrix, swapping the initial tree with TBR (tree bisection and reconnection) and retaining a maximum of one tree in each replicate. Subsequently, all optimal trees were swapped using TBR, holding a maximum of 20,000 trees. To evaluate the relative support for the individual clades, a bootstrap analysis (Felsenstein, 1985) was performed using a total of 10,000 replicates. As the MP trees were similar to the BI trees, we only present the Bayesian results but report bootstrap values (BS) over 50.

To analyse the cpDNA and nrDNA data we followed the procedure described by Wang, Li, and Chen (2014). First, we visually identified significant incongruences between the phylogenies obtained from the cpDNA and nrDNA sequences, and determined the conflicting taxa. The incongruences were tested by applying the SH test (Shimodaira & Hasegawa, 1999) performed under Maximum likelihood (ML) in RAxML v. 8.2.4 (Stamatakis, 2014). Searches of constrained topologies were conducted with 1000 replicates using the models mentioned above. Significant differences between the best ML unconstrained and constrained trees were determined using 10,000 bootstrap replicates and rejecting the hypothesis when $P < 0.01$. In addition, incongruences between ITS and cpDNA data were also visualized in a filtered supernetwork calculated with SplitsTree 4.13.1 (Huson & Bryant, 2006). We used 1,000 Bayesian posterior trees of the nuclear and the plastid dataset, and filtered the splits to show only those present in a minimum of 35% of the input trees. The cycles in the network represent conflicting phylogenetic signals (Huson & Bryant, 2006).

To reconstruct the phylogeny, we performed a combined analysis of the cpDNA and nrDNA datasets. The

combined data matrix was analysed following the same methodology described above (BI and MP). Also, to explain the incongruences or to provide possible interpretations, we performed a coalescence based method to estimate a species tree. Coalescence based-methods can distinguish incomplete lineage sorting (ILS) from hybridization testing whether patterns of incongruence are random (ILS) or non-random (hybridisation – Buckley, Cordeiro, Marshall, & Simon, 2006). The multispecies coalescent model (Xu & Yang, 2016) was used implementing BEAST v2.4.4 (Bouckaert et al., 2014). All nucleotide substitution models were unlinked across loci, and an uncorrelated log-normal clock model was assigned to each sampled locus. We set separate tree models for the chloroplast and the nuclear dataset. Then, we applied the piecewise function with constant root population, and the Yule model for species tree prior. Four runs were conducted in BEAST using 20 million generations and sampling every 5,000. Effective sample size (ESS) >200 was checked in Tracer v. 1.5.0 software, and the first 25% of each run was discarded as burn-in. Replicates were combined using LogCombiner v.1.7.5 (http://beast.bio.ed.ac.uk/LogCombiner), and the species maximum clade credibility tree (MCCT) was calculated using TreeAnnotator v1.5.3 (http://beast.bio.ed.ac.uk/TreeAnnotator).

The final data matrix for the combined and coalescent analyses and the supernetwork graph contained 56 taxa because the incongruent taxa *Bulnesia arborea* (see Results and Discussion below) was coded twice: once as a cpDNA-only accession (nrDNA characters were scored as missing), and once as a nrDNA-only accession (cpDNA characters were scored as missing).

Geographic distribution and ecoregions

To gain a better knowledge of the regions and habitats where the Larreoideae species are distributed in South America we downloaded the occurrence data of each species from the GBIF website (http://www.gbif.org/). Each data point was verified, to discard incomplete, dubious, and/or poorly georeferenced data. The points were subsequently overlapped with the map of Terrestrial Ecoregions from Olson et al. (2001). The results are shown in Table S1 (see supplemental material online).

As the Larreoideae species characterize hot dry regions, we used the free software Quantum GIS 2.10 Pisa (http://www.qgis.org/es/site/) to extract the aridity values of the complete Larreoideae dataset. This allowed us to define the climate classes wherein the Larreoideae species occur (results shown in Figs 1, 2). Climate classes were classified according to the Global Aridity Index (GAI) which was obtained from the CGIAR-CSI GeoPortal (http://www.csi.cgiar.org; Trabucco & Zomer, 2009) at the highest resolution (30 arc seconds or 1 km at equator). It represents the relationship between mean annual precipitation and mean annual potential evapotranspiration. Higher GAI values indicate more humid conditions and lower GAI values more arid conditions.

Results

Chloroplast analyses

The results of the chloroplast analyses are shown in Fig. S1 (see supplemental material online). The length of the *rbcL* and *trnL-F* matrix was 2325 bp (*rbcL* 1402 bp and *trnL-F* 923 bp) and contained a total of 55 taxa. The analyses showed the Larreoideae subfamily as a strongly supported monophyletic group, which appears as sister to the Zygophylloideae subfamily. In both analyses all genera are monophyletic and well supported, with the exception of *Bulnesia* that appears polyphyletic. *Bulnesia* is divided in two groups that are well supported in both the MP and BI analyses. One group, hereafter referred to as the *Bulnesia s.s.* clade, is composed of *B. chilensis*, *B. retama*, *B. foliosa*, and *B. schickendantzii*. These species form a clade with the monotypic genera *Pintoa* and *Metharme*. Further, this clade is sister to the *Larrea* clade. The relationship amongst the *Larrea* members agrees with the section *Bifolium* (*L. tridentata*, *L. cuneifolia*, and *L. divaricata*) and the section *Larrea* (*L. ameghinoi* and *L. nitida*) also found by Lia et al. (2001). The second *Bulnesia* group, composed of *B. arborea*, *B. bonariensis*, and *B. sarmientoi* – hereafter referred to as *Gonopterodendron* clade – appears as sister to *Plectrocarpa*. Additionally, both analyses (MP and BI) support that *Guaiacum* and *Porlieria* form a clade that is sister to *Gonopterodendron* and *Plectrocarpa*.

Nuclear analyses

The results of the nuclear analyses are shown in Fig. S2 (see supplemental material online). The length of the nuclear matrix was 930 bp and contained 33 taxa. The analyses based on nuclear DNA sequences also support the monophyly of Larreoideae and its sister group relationship with Zygophylloideae. As in the chloroplast analyses, all genera are monophyletic and well supported, except *Bulnesia* and *Larrea*, which are polyphyletic. *Bulnesia arborea* appears, in both the MP and the BI analyses, within the *Larrea* clade as part of the section *Bifolium*. The monotypic genus *Metharme* is sister to the *Larrea* clade.

The remaining *Bulnesia* species form a clade, divided into two subclades; the *Bulnesia s.s.* clade (*B. retama*, *B. foliosa*, and *B. schickendantzii*), and the

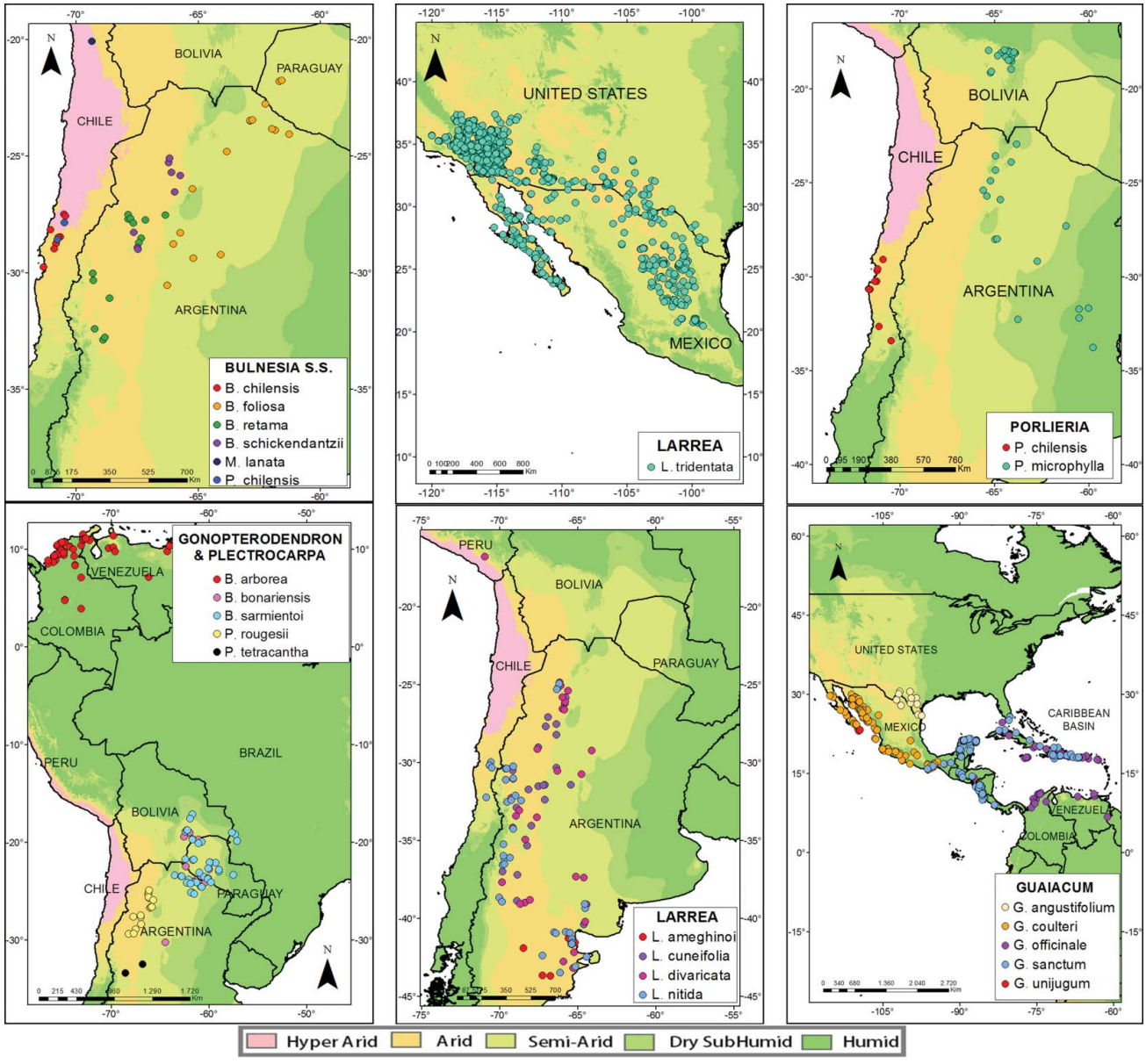

Fig. 1. Geographic distribution of Larreoideae clades. Each clade is composed by different species represented by dots of different colours. Climates class are represented in each map, based on the Global Aridity Index (Trabucco & Zomer, 2009).

Gonopterodendron clade (*B. bonariensis*, and *B. sarmientoi*), that also includes *Plectrocarpa rougesii*.

Finally, the genera *Guaiacum* and *Porlieria* constitute a basal grade within the subfamily.

Three major conflicts were identified between the cpDNA and nrDNA data:

(1) In the chloroplast analyses, *Bulnesia arborea* is part of the *Gonopterodendron* clade while in the ITS gene trees, this species appears within the *Larrea* clade, compromising the monophyly of *Larrea*. The sequences coded as described in Wang *et al*. (2014 – see Materials and Methods) allowed us to identify the placement of the conflicting taxa accessions. For details see the combined and coalescent analyses and the supernetwork graph (Figs 2, Figs S3, S4, see supplemental material online).

(2) In the chloroplast gene trees *Bulnesia* is clearly polyphyletic. In the ITS analyses, if *B. arborea* is not considered, *Bulnesia* appears paraphyletic (including *Plectrocarpa*). The hypothesis of monophyly of *Bulnesia* in the ITS gene tree (excluding *Plectrocarpa*) was significantly rejected by the SH test (difference of log-likelihoods = −20.255316, SD: 7.841962, $P < 0.01$). Similarly, by forcing

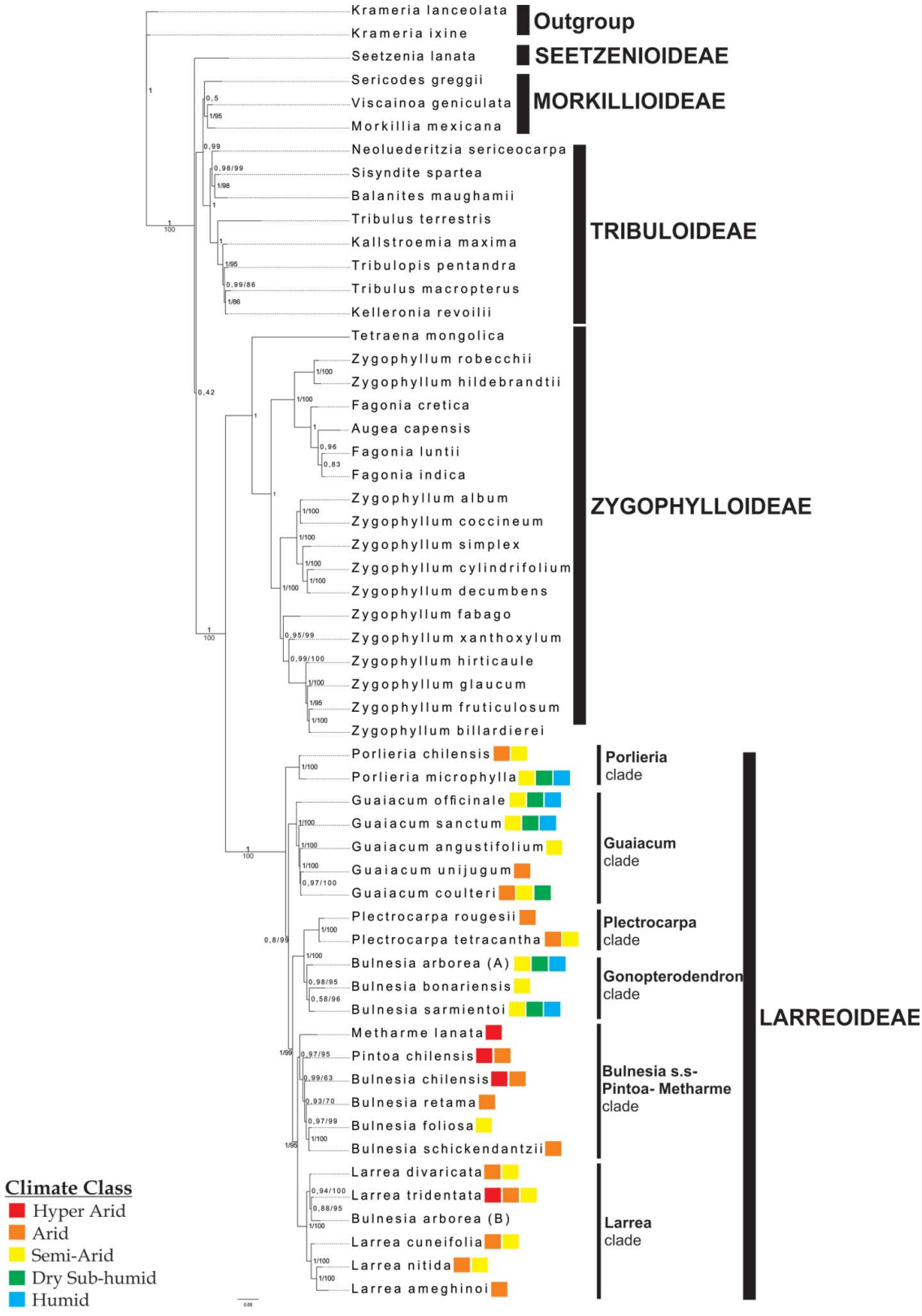

Fig. 2. Bayesian analysis of combined sequence data (rbcL, trnL-F, ITS) of Larreoideae species and allied subfamilies. Numbers above branches indicate the Bayesian probabilities values. Numbers below branches indicate the bootstrap values (only values >50 are reported). Climate classes are indicated for each Larreoideae species (see key). (A) Voucher of *Bulnesia arborea* from Sheahan and Chase (1996, 2000); (B) Voucher of *B. arborea* from Lia et al. (2001).

monophyly of *Bulnesia* in the cpDNA phylogeny led to a significant decrease of likelihood (SH test, difference of log-likelihoods = −149.169519, SD: 23.422428, $P < 0.01$).

(3) In the chloroplast phylogeny, *Guaiacum* and *Porlieria* form a clade that is sister to *Gonopterodendron* and *Plectrocarpa*. In the ITS phylogeny, *Guaiacum* and *Porlieria* constitute a basal clade, sister to the rest of the subfamily. This conflict was resolved in the combined analyses (agreeable with the nrDNA topology). However, in the species tree the solution agreed with the topology supported in the cpDNA analyses.

Combined analyses (chloroplast and nuclear markers)

The results of the combined analyses are shown in Fig. 2. The length of the combined matrix was 3256 bp (*rbcL* 1402 bp, *trnL-F* 924 bp, and ITS 930 bp) and contained a total of 56 taxa. Both the MP and the BI analyses showed the Larreoideae subfamily as a strongly supported monophyletic group. Once again *Bulnesia* is recovered as polyphyletic while all remaining genera are monophyletic and well supported. The *Gonopterodendron* clade is sister to *Plectrocarpa* and the *Bulnesia s.s.* form a clade with the monotypic genera *Pintoa* and *Metharme*, sister group to the *Larrea* clade. *Guaiacum* and *Porlieria* constitute a basal grade within the subfamily.

The combined cpDNA + nrDNA dataset resolves most of the conflicts between the cpDNA and nrDNA datasets in favour of the cpDNA topology (the only exception being the placement of *Guaiacum* and *Porlieria* – Fig. 2). In the supernetwork, *Bulnesia* also appears split into two groups clearly differentiated: *Bulnesia s.s*. and *Gonopterodendron* (Fig. S4, see supplemental material online). Furthermore, the supernetwork graph clearly showed the conflicting placement of *Bulnesia arborea* while the placement of *Guaiacum* and *Porlieria* was solved in favour of the cpDNA topology (Fig. S4, see supplemental material online). The species tree is congruent with all results showed by the supernetwork (Fig. S3, see supplemental material online).

Taxonomic treatment

Here, based on evidence from the phylogenetic analyses of the molecular data described above (Figs 2, S1, S3, S4, see supplemental material online), we propose dividing the actual *Bulnesia* into two genera: *Bulnesia s.s.* (Fig. 3) and *Gonopterodendron* (Fig. 4).

Bulnesia Gay
(Fig. 3), *Hist. Fis. Pol. Chile Bot. 1*: 474 (1845)

Type species: *Bulnesia chilensis* Gay, *Hist. Fis. Pol. Chile Bot. 1*: 475 (1845).

ETYMOLOGY: The name *Bulnesia* is given in honour of General Manuel Bulnes.

DIAGNOSIS: Actinomorphic flowers, less than 25 mm in diameter, yellow, orange-yellow petals. Stamens equal to each other. Slightly developed carpophores from 0.1 to 1.2 mm. Mericarps smaller than 30 mm long. Albuminous, oblong-reniform seeds, lenticular, elliptical or oval transverse section, less than 11 mm long × 3.5 mm lat.; nitid tuberculate testa, coriaceous, firmly attached to the endosperm. Oblong cotyledons. Alternate leaflets, subopposite to almost perfectly opposite. Shrubs.

≡ *Gonoptera* Turcz, *Bull. Soc. Imp. Naturalistes Moscou* 20: 150 (1847). Type species: *Gonoptera chilensis* Turcz, *Bull. Soc. Imp. Naturalistes Moscou* 20: 151 (1847).

Bulnesia chilensis Gay
(Fig. 3.1), *Hist. Fis. Pol. Chile Bot. 1*: 475 (1845).

Type species: Chile, *C. Gay s.n.* (lectotype, G00342498! here designated).

≡ *Gonoptera chilensis* Turcz*, Bull. Soc. Imp. Naturalistes Moscou* 20: 151 (1847). Type species: Chile, Concepcion, *T.C. Bridges 1303* (holotype, K000531337!)

Note: Gay's material is supposed to be deposited at the Muséum National d'Histoire Naturelle (Stafleu & Cowan, 1979). However, the holotype is not found here (Marc Jeanson, personal communication, 29 Aug. 2017). The specimen found in K (K000531339!) is a duplicate of the collection found in Paris, as is indicated by the specimen label, so it could be designated as a lectotype. However, this specimen lacks leaves, flowers, and fruits material. Therefore, the sheet G00342498! is here designated as a lectotype being the most complete.

Bulnesia foliosa Griseb.

(Fig. 3.2), *Abh. Königl. Ges. Wiss. Göttingen 19*: 106 (1874). Type species: Argentina, Santiago del Estero, 4 Dec. 1871, *P.G. Lorentz 11* (lectotype GOET008946! designated by Palacios & Hunziker, *Darwiniana* 25: 304 (1984), isolectotypes CORD00005906!, G00342497!)

Bulnesia retama (Gillies ex Hook. & Arn.) Griseb.

(Fig. 3.3), *Abh. Königl. Ges. Wiss. Göttingen* 19: 106 (1874) ≡ *Zygophyllum retama* Gillies ex Hook. & Arn., *Bot. Misc*. 3: 166 (1833). Type species: Argentina, 'From Mendoza to San Juan, 2000-3000 feet, Nom. vern. Retama', *J. Gillies s.n. [70]* (holotype, K000531329!; isotype, E00285664!)

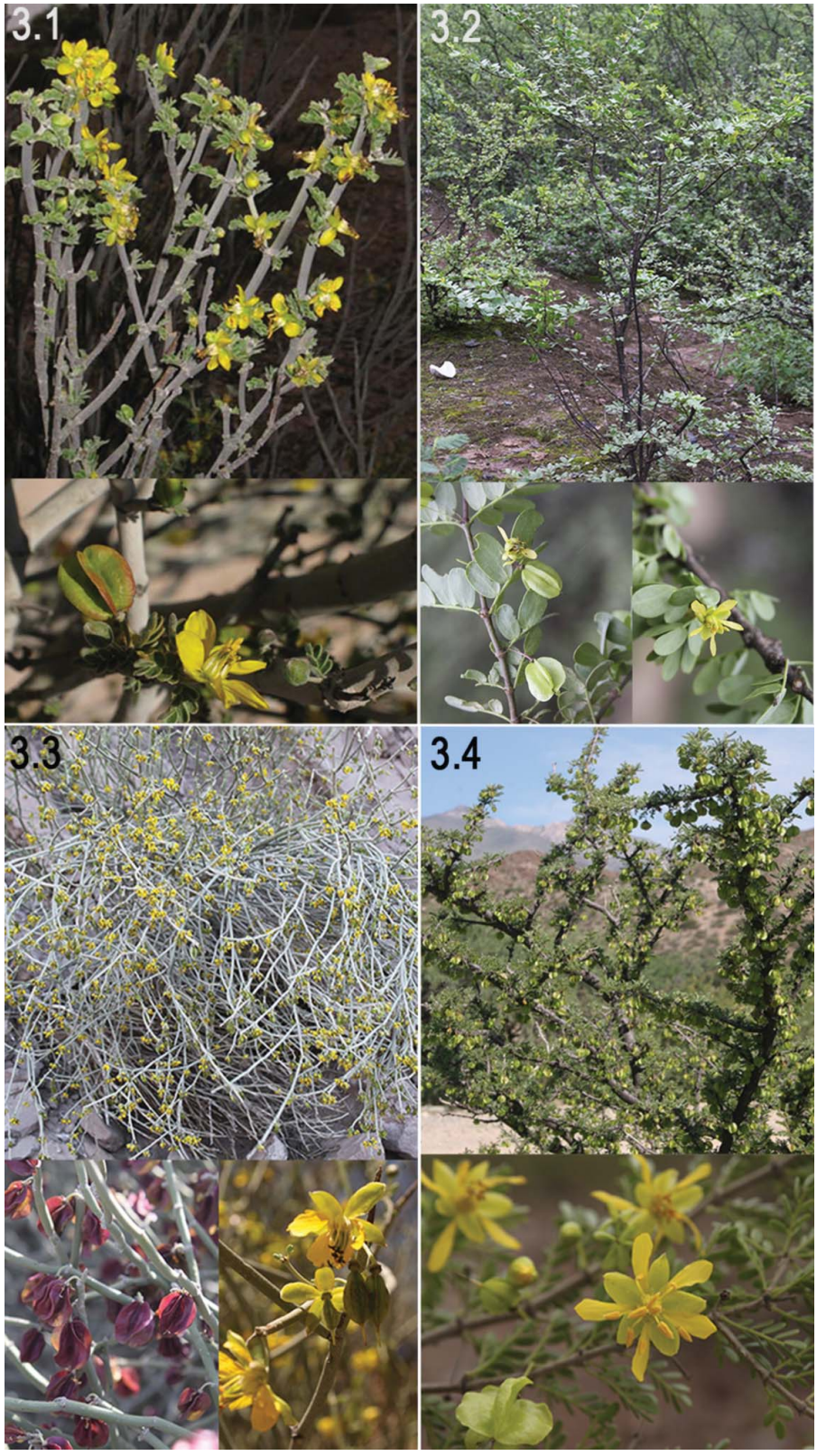

Fig. 3. The *Bulnesia* genus: 1) *Bulnesia chilensis,* photos by Michael Belov 2006 and 2008 (www.chileflora.com); 2) *Bulnesia foliosa,* photo by Jose F. Pensiero (http://www.fca.unl.edu.ar/prodocova/IRUPE/index); 3) *Bulnesia retama,* photos from SI (www.floraargentina.edu.ar); and 4) *Bulnesia schickendatzii,* photos from SI.

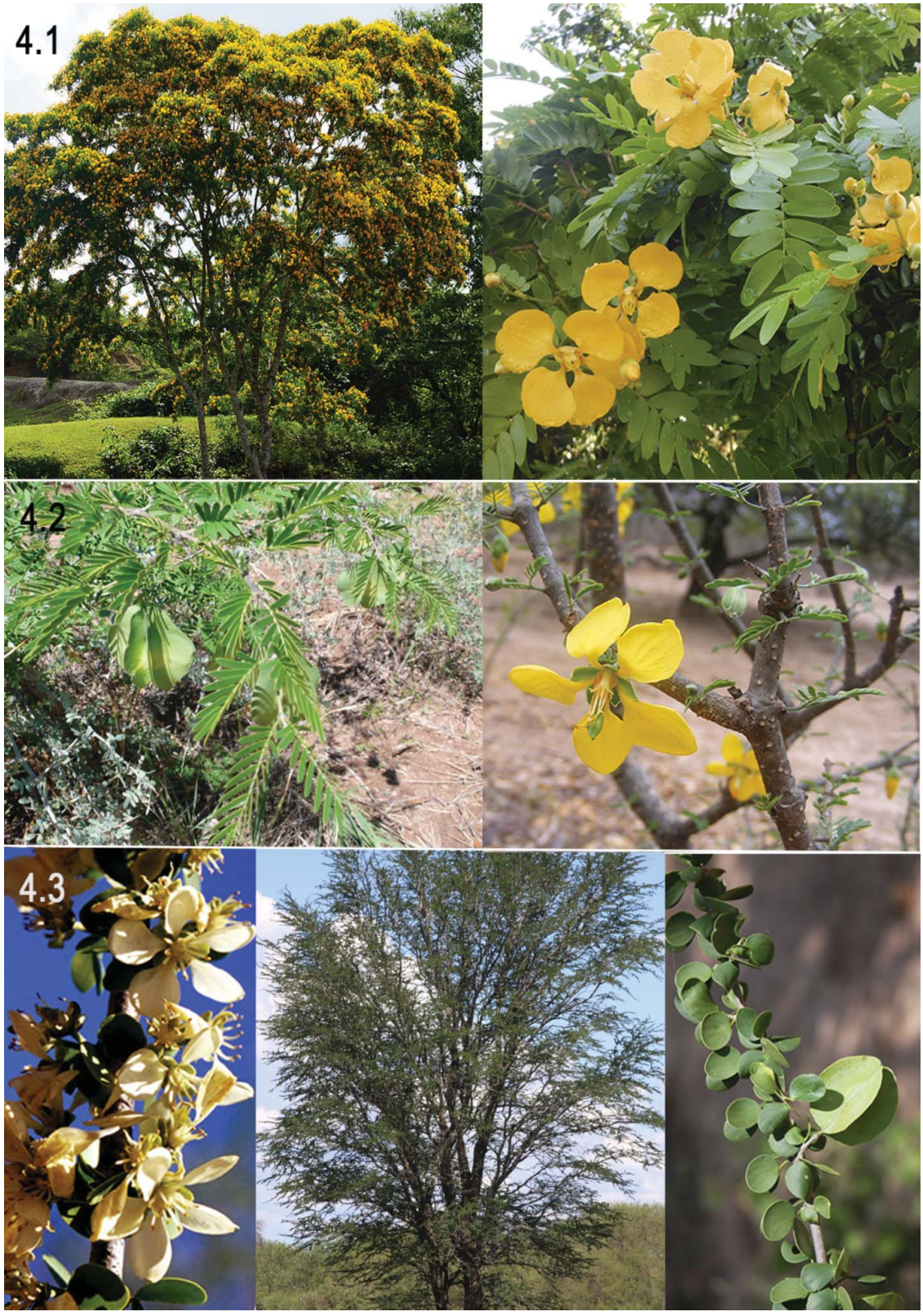

Fig. 4. The *Gonopterodendron* genus: 1) *Gonopterodendron arboreum,* photos by Richards Lyons Nursery (www.richardlyonsnursery.com); 2) *Gonopterodendron bonariensis*, photos by Koya Akulova (http://calphotos.berkeley.edu/ - left) and A. Grau (right); 3) *Gonopterodendron sarmientoi*, photos by J. Pensiero.

≡ *Bulnesia macrocarpa* Phil., *Sert. Mendoc. Alt.* 9: 167 (1870). Type species: Argentina. Mendoza, 1868/69, *P. Ortega s.n.* (lectotype SGO000002960!, designated by Godoy-Bürki, *Phytotaxa* 239: 293 (2015), isolectotype SGO000002959!)

Bulnesia rivas-martinezii G. Navarro, *Novon 4*: 280. (1994).

Type species: Bolivia. Dpto. Chuquisaca. Prov. Sud Cinti, between Villa Abecia and Camargo, at 1 km from Saladillo. 28 Dec. 1992, *G. Navarro 1913* (holotype, LPB0000851!, isotype MO-251168!)

Bulnesia schickendantzii Hieron. ex. Griseb. (Fig. 3.4), *Abh. Königl. Ges. Wiss. Göttingen 24*: 75. (1879).

Type species: Argentina, Prov. Catamarca, Loma de Belén, Nov. 1873, *F. Schickendantz 261* (holotype, GOET008947!, isotype, CORD00005908!).

Gonopterodendron (Griseb.) A.C. Godoy-Bürki, stat. nov.
(Fig. 4)

≡ *Bulnesia* sect. *Gonopterodendron* Griseb. *Abh. Königl. Ges. Wiss. Göttingen* 24: 75 (1879) ≡ *Bulnesia* subgen. *Gonopterodendron* (Griseb.) R.A. Palacios & J.H. Hunz. *Darwiniana* 25: 312 (1984). Type species: *Bulnesia sarmientoi* Lorentz ex. Griseb, *Abh. Königl. Ges. Wiss. Göttingen* 24: 75 (1879).

ETYMOLOGY: *Gonia*, Greek, means angle; *pteron*, Greek, means wing, and *dendron*, Greek, means tree.

DIAGNOSIS: Flowers generally larger than 25 mm in diameter, some zygomorphic (except *G. sarmientoi*), with yellow, orange-yellow or whitish petals. Stamens unequal, the upper 3 modified with respect to the remaining 7 (except *G. sarmientoi*). Carpophores well developed from 4 to 9 mm. Mericarps larger than 32 mm long × 26 mm lat. Ex-albuminous, semi-circular or hemi-elliptic seeds, linear section and a rough tenuous testa of spongy-pruinouse appearance, greater than 9 mm long × 6 mm lat. semi-circular to hemi-elliptic cotyledons. Leaflets always perfectly alternate. Trees or rarely shrubs.

Gonopterodendron arboreum (Jacq.) A.C. Godoy-Bürki, comb. nov.
(Fig. 4.1)

≡ *Zygophyllum arboreum* Jacq., *Select. Stirp. Am. Hist*. 130, tab. 83 (1763) ≡ *Bulnesia arborea* (Jacq.) Engl., *Nat. Pflanzenf*. 3 Abt. 4: 84 (1890). Type species: 'Colombia, habitat Carthagenae in vallibus fylvaticis and paffim in fylvis areno fis maritimis', lectotype tab. 83, *Select. Stirp. Amer. Hist*., 1763, here designated.

Note: Jacquin's material is supposed to be deposited at the Natural History Museum of Vienna (Stafleu & Cowan, 1979). However, the holotype is missing, as was mentioned by Palacios and Hunziker (1984), and confirmed by the curator of W, Ernst Vitek (personal communication, 29 Aug 2017). Therefore, the iconography accompanying the original description of the species is here designated as the lectotype because identification of the species from the image is univocal.

Gonopterodendron bonariensis (Griseb.) A.C. Godoy-Bürki, comb. nov.
(Fig. 4.2)

≡ *Bulnesia bonariensis* Griseb., *Abh. Königl. Ges. Wiss. Göttingen* 19: 105 (1874). Type species: Argentina, Santiago del Estero. Hauptbestandtheil der Buschvegetation hinter Ojo de Agua, hinter Loreto, etc., Dec. 1871, *P.G. Lorentz 26* (lectotype, GOET008945!, designated by Palacios & Hunziker, *Darwiniana* 25: 318 (1984); isolectotypes CORD00005904!, CORD00005905!, G00342499!).

Gonopterodendron carrapo (Killip & Dugand) A.C. Godoy-Bürki, comb. nov.

≡ *Bulnesia carrapo* Killip & Dugand, in Dugand (1944), *Caldasia* 3: 35 (1944). Type species: Colombia, Depto. Cundinamarca, Hacienda 'El Cucharo', between Tocaima and Pubenza, 380–400 a.s.l. 8 May 1944, *E. P. Killip, A. Dugand & R. Jaramillo 38374* (lectotype, COL000001828!, here designated; isolectotypes, A00043906!, COL000001827!, COL000001829!, COL000001830!, COL000206522!, F0074915F!, G00342500!, NY00388419!SP002947!, S08-11862!, S-R-9917!, US00101297!).

Note: In the protologue of *B. carrapo* the authors cited as holotype the specimen Killip *et al*. 38374 deposited in COL (Herb. Nac. Colombia). However, we found five well-conserved specimens (catalogued as isotypes) coinciding with the type collection. To avoid misinterpretation, we designated here as lectotype COL000001828! for being most similar to the iconography accompanying the protologue, and for being the most complete herbarium specimen.

Gonopterodendron sarmientoi (Lorentz ex Griseb.) A.C. Godoy-Bürki comb. nov.
(Fig. 4.3)

≡ *Bulnesia sarmientoi* Lorentz ex Griseb., *Abh. Königl. Ges. Wiss. Göttingen* 24: 75 (1879). Type species: Argentina [Salta]: Dragones. Gr. Chaco ad fl. Bermejo, August 1873, *P. G. Lorentz & G. Hieronymus 576* (lectotype, GOET008963!, designated by Godoy-Bürki, *Phytotaxa*

239: 293 (2015); isolectotypes BAF00000191!, BAF00000192!, BAF00000193!, CORD00005909!, CORD00005910!, GOET008964!).

Geographic distribution and ecoregions

The results are shown in Fig. 1, and Fig. 2, Table S1 (see supplemental material online) and discussed below. Table S1 shows the biomes and ecoregions where the Larreoideae species presently occur. Fig. 1 shows the geographic distribution of the Larreoideae clades in combination with the GAI values (expressed as climate classes). In Fig. 2, we added, to the phylogeny, the climate classes in which each of the Larreoideae species occur.

Discussion

Our results support the monophyly of the American subfamily Larreoideae as well as its position as sister to the subfamily Zygophylloideae as found in earlier studies (Bellstedt et al., 2012; Sheahan & Chase, 2000; Wu et al., 2015). All genera with the exception of *Bulnesia* were found to be monophyletic (Fig. 2).

The two major conflicts between the ITS and cpDNA tree topologies concern the position of the *Bulnesia* species. One conflict involves the position of *Bulnesia arborea*. Morphologically, and according to chloroplast analyses (Fig. S1, see supplemental material online), this species clearly belongs to the *Gonopterodendron* clade. However, in the nuclear analyses (Fig. S2, see supplemental material online) *Bulnesia arborea* is included in the *Larrea* clade together with *L. tridentata*, *L. divaricata*, and *L. cuneifolia*, all species well known for their capability of hybridization (Hunziker & Comas, 2002; Hunziker, Palacios, Valesi, & Poggio, 1978; Lia et al., 2001; Yang, Hunziker, Poggio, & Naranjo, 1977). Phylogenetic incongruence between two gene trees may emerge from several processes such as paralogy, hybridization, or incomplete lineage sorting. In the present case, events of reticulate evolution are the least probable cause because *Bulnesia arborea* does not exist in sympatry with any of the *Larrea* species (Fig. 1). On the other hand, the sequences available of *B. arborea* from GenBank correspond to different voucher material. The *rbcL* and *trnL-F* sequences were obtained by Sheahan and Chase (1996, 2000) while the ITS sequence was obtained from a different voucher by Lia et al. (2001). This last sequence is 99% identical to the sequence of *L. tridentata* when subjected to a BLAST search in the National Centre for Biotechnology Information database (http://www.ncbi.nlm.nih.gov). Therefore, it seems possible that the origin of incongruence is an analytical artefact (such as a laboratory error – Fig. 2, Figs S3, S4, see supplemental material online).

The second major conflict concerns the polyphyly of genus *Bulnesia*. Our cpDNA phylogeny supports two divergent lineages of *Bulnesia* that appear as polyphyletic (Fig. S1, see supplemental material online). These two lineages persist in the nrDNA phylogeny where they form a clade that also includes *Plectrocarpa rougesii*, rendering *Bulnesia* paraphyletic (Fig. S2, see supplemental material online). The coalescent based approach does not allow us to explain this incongruence, as more than two unlinked genomic datasets are required to distinguish between ILS and hybridization (Buckley et al., 2006) but shows *Bulnesia* as polyphyletic (Fig. S3, see supplemental material online). Hybridization has been demonstrated within the genera *Larrea* (Hunziker & Comas, 2002; Hunziker et al., 1978; Lia et al., 2001; Yang et al., 1977) and *Guaiacum* (Dertien & Duvall, 2014), and is therefore a likely hypothesis for explaining incongruence between the plastid and ITS phylogenies. Otherwise, despite the above-mentioned conflicts, our results showed that the hypothesis of monophyly of *Bulnesia* is rejected. The combined species tree and the supernetwork also agree with this statement (Fig. 2, Fig. S4, see supplemental material online).

Grisebach (1879) was the first to propose a division of the genus *Bulnesia* (section *Gonopterodendron* – section *Bulnesia*). Yet, some disagreement arose around this subdivision (Descole & O'Donell, 1943; Descole et al., 1940). Later, Palacios and Hunziker (1984) revised *Bulnesia* and found important differences in the seed endosperm, and other morphological features that sustained its division into two subgenera which were corroborated by Comas et al. (1998). Our molecular phylogenetic analyses recovered *Bulnesia* as a polyphyletic group, providing enough support for separating this taxon into distinct genera (Fig. 2, Figs S1, S3, and S4, see online supplemental material). We propose to redefine the genus *Bulnesia* as two genera: *Bulnesia s.s.* and *Gonopterodendron* (see the Results section for details of the taxonomic treatment). The *Bulnesia s.s.* (Fig. 3) includes *Bulnesia chilensis* (type species of *Bulnesia* – Fig. 3.1), *B. foliosa* (Fig. 3.2), *B. retama* (Fig. 3.3), *B. rivas-martinezii*, and *B. schickendantzii* (Fig. 3.4). The subgenus *Gonopterodendron* (Fig. 4) is here established as genus, and includes *Bulnesia arborea* (Fig. 4.1) *B. bonariensis* (Fig. 4.2), *B. carrapo*, and *B. sarmientoi* (Fig. 4.3). We did not include in our analyses *Bulnesia carrapo* and *Bulnesia rivas-martinezii* for which material could not be obtained. However, we believe that *Bulnesia rivas-martinezii* should be maintained in *Bulnesia s.s.* (judged by morphological similarity – Navarro, 1994), and *Bulnesia carrapo* should be transferred to *Gonopterodendron*. The latter is supported by morphological characters shared by the members of *Gonopterodendron* (Palacios & Hunziker, 1984), and by biochemical studies that show *B. carrapo* as sister to *B. arborea*, forming a clade with *B. bonarienses* and

B. sarmientoi in agreement with the subdivision of the genus (Comas et al., 1998).

The *Porlieria* clade: In the combined analyses a dichotomy divides genus *Porlieria* from the remainder of the species of the subfamily (Fig. 2). Two species of *Porlieria* were evaluated: *P. chilensis* and *P. microphylla*, found respectively on the western and eastern side of the Andes in southern South America (Zuloaga, Morrone, & Belgrano, 2008 – Fig. 1). *Porlieria chilensis* is a shrub endemic to Chile where it is found in patches in arid and semi-arid environments from the north-east of Coquimbo (Region IV) to the northern limit of O'Higgins (Region VI – Loayza, Rios, & Carvajal, 2015 – Fig. 1). This species inhabits the Chilean Matorral, and is the only Chilean Larreoideae species that is not found in hyper-arid locations (Fig. 1, Table S1, see supplemental material online). *Porlieria microphylla* is a shrub found in semi-arid to humid environments of Argentina, Uruguay (Zuloaga et al., 2008 – Fig. 1), and Bolivia (Jörgensen, Nee, & Beck, 2014 – Fig. 1).

The *Guaiacum* clade: The second dichotomy within the family separates the Mesoamerican genus *Guaiacum* from the remainder of the family (Fig. 2). *Guaiacum* is the only genus of the subfamily that does not occur in South America (Fig. 1). The genus is found in arid to humid regions of the Caribbean basin and Mexico (Fig. 1, Table S1, see supplemental material online).

Vail (1895) suggested submerging *Porlieria* into *Guaiacum* based on their morphological similarities. Our combined study showed that both *Guaiacum* and *Porlieria* are monophyletic (Fig. 2) and do not overlap in their geographic distributions (Fig. 1). Thus, we suggest that the two genera should remain apart as separate entities. Nonetheless, the placement of both *Guaiacum* and *Porlieria* is still unsolved. The combined cpDNA+nrDNA phylogeny (Fig. 2) is resolved in favour of the ITS analyses (Fig. S2, see supplemental material online) while the species trees and the supernetwork (Figs S3, S4, see supplemental material online) are consistent with the plastid analyses (Fig. S1, see online supplementary material). We believe that a complete sampling of *Porlieria* (we analyse 2 of 4 species) could define this incongruence.

The rest of the subfamily Larreoideae occurs in a nearly all Southern Cone clade in which only *Bulnesia arborea* and *Larrea tridentata* are found elsewhere (Fig. 1, Table S1, see supplemental material online).

The *Bulnesia s.s.–Pintoa–Metharme–Larrea* clade: the *Bulnesia* species included in this clade (*B. chilensis*, *B. foliosa*, *B. retama*, and *B. schickendantzii*) constitute the subgenus *Bulnesia* recognized by Palacios and Hunziker (1984) on the basis of shared morphological characters, such as albuminous seed and other morphological characteristics e.g., habit, fruit and seed size, flower symmetry, and seed shape. *Bulnesia s.s.* grouped with the monotypic genera *Metharme* and *Pintoa.* The *Bulnesia s.s.–Pintoa–Metharme* clade is sister to the *Larrea* clade (Fig. 2).

Metharme lanata, and *Pintoa chilensis*, together with *Bulnesia chilensis* are all species found in Chile (Fig. 1). The three species occur in environments with fluctuating temperatures and whose precipitation values do not exceed 250 mm $year^{-1}$ and, in the more severe cases, do not even surpass 1 mm $year^{-1}$. These species inhabit, together with the non-South American *Larrea tridentata*, the most arid environments occupied by any members of the Larroideae subfamily (Fig. 1). These environments are found in the Atacama Desert, the northern portion of the Chilean matorral, and in the Chihuahuan, Sonoran, and Mojave deserts (for more details see Table S1, see supplemental material online). *Metharme lanata* seems to be the most specialized species as it is restricted to the Atacama Desert with less than 1 mm $year^{-1}$ (Teillier, 2001); however there are no morphological studies on the adaptive characters that allow the species to persist in this environment. The opposite is true for *Larrea tridentata* where several studies have reported on adaptive characters such as a strong seasonal acclimatory response to high temperature and water deficit, resulting in a remarkable tolerance to temperature extremes (Mooney, Björkman, & Collatz, 1978; Smith, Monson, & Anderson, 1997). Further, this species appears to be more drought tolerant than its closest relatives, the South American *Larrea* species (Fig. 1, Table S1). *Larrea divaricata*, *L. cuneifolia*, and *L. nitida* are found in arid and semi-arid environments in Argentina, Chile, Bolivia, and Peru (Fig. 1), mainly in the Monte Desert and in the ecotone between the Monte and the Patagonian steppe (Ezcurra, Montana, & Arizaga, 1991; Table S1, see supplemental material online).

The remaining *Bulnesia* species are found in environments similar to those of their Chilean relatives but with higher rainfall and less abrupt temperature changes. These species are found in arid and semi-arid habitats of Argentina (*B. foliosa*, *B. retama*, and *B. schickendanzii*) and Paraguay (*B. foliosa*) such as the Dry Chaco and the Monte Desert (Fig. 1; Table S1, see supplemental material online), the last being the driest rangeland of the Southern Cone (Elías & Aagesen, 2016; Fernández & Busso, 1999).

The *Gonopterodendron–Plectocarpa* clade: the *Bulnesia* species in this clade (*B. arborea*, *B. bonariensis*, and *B. sarmientoi*) comprise the subgenus *Gonopterodendron* recognized by Palacios and Hunziker (1984). The species of this group share the presence of exalbuminous seeds and an arboreal habit. According to Palacios and Hunziker (1984), the more specialized structures of the flowers and seeds in *Gonopterodendron* indicate a more recent evolution of the group, although we do not find evidence of this by inspecting the length of the branches in the phylogenetic tree.

Bulnesia sarmientoi is mostly found in humid to semi-arid regions of the Great South American Chaco while *Bulnesia bonariensis* is exclusively found in dry sub-humid forests of Argentina or Bolivia (Fig. 1, Table S1, see supplemental material online). *Bulnesia arborea*, the northernmost species of the genus, is distributed in Colombia and Venezuela in areas that receive between 600 to 1500 mm of precipitation (Fig. 1). *Plectocarpa* consists of two species; *P. tetracantha* and *P. rougesii*, both distributed in arid regions of Argentina (Fig. 1, Table S1, see supplemental material online). In the ITS tree *Plectrocarpa* is nested within *Gonopterodendron* (Fig. S2, see supplemental material online) so it could be interpreted that the former should be reduced to the latter. However, before submerging *Plectrocarpa* into *Gonopterodendron*, the ITS analysis should be completed including the sequence of *Plectrocarpa tetracantha*. Furthermore, *Plectrocarpa* is morphologically very different from *Gonopterodendron*; the species has a shrubby habit, with thorns along its branches, and smaller flowers and fruits. In conclusion, until proven otherwise, we maintain *Plectrocarpa* as a genus distinct from *Gonopterodendron.*

Conclusions

Judged by the estimated age (Wu et al., 2015) and present distribution of Larreoideae (Fig. 1, Table S1, see supplemental material online), we believe that its evolution may have occurred during a period that became progressively arid, as occurred with related subfamilies in Africa (Bellstedt et al., 2012) and in Asia (Wu et al., 2015). During the Oligocene and the Miocene, there was a global trend of decreasing temperature that generated a major environmental change in southern South America where all biogeographic regions migrated to lower latitudes (Iglesias, Artabe, & Morel, 2011). Furthermore, there was an expansion of xeric forms that increased in diversity and abundance mainly during the late Miocene (Barreda & Palazzesi, 2007; Palazzesi & Barreda, 2012). The rise of the Andes accelerated this process with the development of extreme, and a more seasonal climate aridity (Antonelli, Nylander, Persson, & Sanmartín, 2009; Barreda & Palazzesi, 2007; Catalano, Vilardi, Tosto, & Saidman, 2008; Palazzesi & Barreda, 2012; Roig, Roig-Juñent, & Corbalán, 2009) that exists at present in the areas where most of the Larreoideae species occur.

The Larreoideae species are currently mainly found in arid and semi-arid environments although humid and hyper-arid regions have also been reached (Fig. 1, Table S1, see supplemental material online). However, the phylogeny in Fig. 2 suggests that presences in these latter environments are derived states within the group, where some species probably have acquired additional characters to adapt to these new conditions. While our study shows that the Larreoideae have adapted to a broad precipitation range (Figs 1, 2; Table S1, see supplemental material online), there are no suggestions of an adaptation to a broad temperature range. *Larrea ameghinoi*, the southernmost species of the group, reaches 43°S where the Monte desert grades into the Patagonian Steppe vegetation (Fig. 1) but not beyond where the mean annual temperature start to decrease. We believe that the Larreoideae species have not adapted to cold environments but only to marked changes in precipitation (from humid to hyper-arid climate, Figs 1, 2), reflecting that aridity in southern South America – at least locally – may be an ancient condition while low temperatures are of much more recent date (Iglesias et al., 2011). Nonetheless, the hypothesis of niche evolution should be tested by quantitative analyses using a phylogenetic framework, which is provided by this study for future macro-ecological studies.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Supplemental data

Supplemental data for this article can be accessed at https://doi.org/10.1080/14772000.2018.1451406

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